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THE MORPHOLOGY AND AFFINITIES OF GNETUM

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1. INTRODUCTION

The Gnetales have been called the lure and the despair of the morphologist. They are alluring because they promise to give a solution to the morphologist's great problem, the origin of the Angiosperms. They are a despair in that heretofore, in spite of many efforts, no one has been able either to establish convincingly or to disprove their Angiospermic connection. Some botanists maintain that they occupy a sort of intermediate position between Angiosperms and Gymnosperms; others believe that they represent a line of evolution which developed parallel to the Angiospermic line from the same ancestral group; others again deny all relationship between the two groups, believing that the undoubted points of resemblance have been independently acquired. Nor is there agreement in regard to their Gymnospermic connection. While most morphologists are agreed that they are the highest of the Gymnosperms some believe that they have been derived from the Bennetitales and others that they have come from the Coniferales.

An obvious contribution toward a solution of these problems would be a thorough investigation of the essential morphology of the genus Gnetum about which our information is very meager. In addition to its own interest and its bearing on general problems of Gnetalean and Angiospermic affinities, it should throw light on other problems such as the morphology of the gametophytic structures and endosperm of Angiosperms. Undoubtedly the morphology of Gnetum would have been thoroughly investigated long ago but for the great difficulty in securing proper material. With our present accumulation of knowledge in respect to the gametophytic conditions and endosperm of almost every other living genus of Gymnosperms

and of representative forms from the whole Angiospermic series, the need of the investigation is all the more urgent.

The present work is an attempt to supply the missing data. In it are described the gametophytes, endosperm, embryo, and those parts of the sporophytic generation concerned with reproduction. In almost all cases the development of the structures concerned is described. Finally the bearing of the results obtained on the general problems such as those just outlined is discussed. The data have been obtained from several species of the genus. Two species representing the extremes of variation have been studied in practically all stages, and parts of the life history of several other species were determined.

2. History

The history of the work on the morphology of the inflorescences and flowers was narrated very clearly and completely in 1913 by Lignier and Tison (17) to whose article the reader is referred. The work on the gametophytic generation prior to 1899 was carefully reviewed by Lotsy (19). As this is the subject with which we are primarily concerned in the present work, the principal publications should be mentioned. They are those of Karsten (12, 13 and 14), of Strasburger (25) and of Bower (5). All these contributions and that of Lotsy give us only fragments of the life history. And since the publication of Lotsy's work in 1899 very few articles have appeared dealing with this phase of the life history. In a further contribution Lotsy (20) claimed that parthenogenesis may occur in *G. ula*. In 1908 Coulter (7) described a mature embryo-sac and some early stages in embryo-formation in *G. gnemon*. In 1912 Pearson (22) described some early stages in the male gametophyte of *G. africanum*.[1]

3. Materials and Collections

The material on which this investigation is based was collected during a visit to the Malay Archipelago in 1913. Most of the collecting was done in the Botanic Garden at Buitenzorg, Java, and in the adjacent country. Collections were also made at several other localities in Java and at Singapore.

[1] Since the present paper went to the printer a more recent article by Pearson has appeared (Jour. Linn. Soc. **43**: 55–65, 1915) in which he described the strobili of Gnetum and the development of the endosperm.

In the famous Botanic Garden at Buitenzorg are excellent speci- mens of several species of the genus. From these was secured almost complete male material of *G. latifolium*, *G. moluccense*, *G. neglectum*, and *G. ula*, as well as of two unnamed species, *G. sp. 33*, and *G. sp. Borneo* of the Garden records.[1] Of female material almost complete stages of *G. moluccense*, *G. neglectum* and *G. sp. 59*, were obtained in the garden. Outside the garden one may find plenty of trees of *G. gnemon* in any native village where they are cultivated for the edible inflorescences and fruits. These trees, however, are almost all fe- male. It appears that the natives destroy the male trees because they do not bear fruit, not knowing that pollination is necessary before fruit will be borne on the female trees. Consequently it is very difficult to secure male material or the fertilization and later stages of the female.[2] By prolonged search, however, I succeeded in finding in two villages (Tjidoerock and Tjipatat) a few male trees in close proximity to female specimens and from these it was easy to obtain the desired stages.

In the nearly impenetrable jungles at the base of Mt. Salak one can secure an abundance of material of *G. funiculare*. In isolated situations in the forest one can rarely find *G. neglectum* and *G. lati- folium*.

With the exception of *G. gnemon* all these species are vines which are strikingly Dicotyledonous in appearance. The wild specimens prefer to climb among the branches of the tallest trees. The lower part of the stem is usually naked for a length of twenty to fifty feet, the leaves occurring only up among the branches of the tree to which the specimen is clinging. Some species have the strap-shaped stems of typical lianas. *G. gnemon* differs from the others in being an erect and often stout tree. It will appear later that this species is also very distinct from the others in its gametophytic generation.

There appears to be no definite flowering season in the case of *G. gnemon*. One may find all stages at almost any time of year.

[1] The whole systematic classification of the genus is in urgent need of revision. I believe this is to be undertaken very soon by Dr. Valeton of the Buitenzorg Garden and consequently I have considered it advisable to retain the old names and numbers of the garden records, pending the publication of Dr. Valeton's study.

[2] It may also be that there is something peculiar in the sex determination of this species, for I have sometimes found groups of young specimens growing wild where they had apparently never been molested, and yet they were almost invariably female.

Of course the individual trees do not flower continuously but may begin to flower at any time. Between the appearance of the strobili and the formation of young green fruit a period of two or three months elapses: In the males as in so many tropical trees there are several flowering periods. One crop of inflorescences appears, matures and falls within a few weeks and within another few weeks another crop appears. The remaining species have more definite flowering seasons. Most of them begin about February in Java but the height of the season is reached in May. Individual specimens may not flower until long after this. Consequently in May and June one may obtain at the same time on different specimens many stages in the life history. When a specimen of *G. ula* begins to flower in February, young fruit with a large endosperm and suspensors will be present in May.

4. INFLORESCENCES

1. *Normal.* (*a*) *Staminate.*—The staminate inflorescences usually appear in the axils of leaves, often in the old axils from which the leaves have fallen. As in the case of vegetative branches, it appears that new inflorescences may develop year after year in the same axil. Often two inflorescences will appear in the same axil at the same time. Frequently they are terminal. Frequently, too, they occur on the old wood of stout stems particularly on the naked parts of the stems of climbing species.

In some species each inflorescence consists of a single strobilus (*G. moluccense*). In others three strobili form a ternately divided inflorescence although the two lateral ones may be suppressed (*G. gnemon*). In others again the whole inflorescence may be profusely branched or paniculate (*G. sp. 33*). The strobilus itself, as is well known, normally consists of an axis bearing a series of collars (connate bracts) in which the flowers are borne attached to the axis. The constituent bracts of the lowermost collar are often distinct. The flowers are numerous and occur massed together in a low spiral above each collar. Invariably above each set of staminate flowers are abortive ovulate ones normally arranged in a single ring. The invariable occurrence of these abortive ovulate flowers in staminate strobili is a fact not sufficiently appreciated. Certain abnormal staminate strobili which appear to have considerable significance are described in connection with similar ovulate ones which are commoner and more easily interpreted.

(b) *Ovulate.*—The ovulate inflorescences, like the staminate, may be axillary, terminal or on old wood. Like the staminate ones, too, they may consist of a single strobilus or be more or less branched and paniculate. For any given species the particular form of the inflorescence is the same in the female as in the male. Each strobilus further resembles the male ones in consisting of a series of cups with flowers in their axils. But the cups are not contiguous as in the male and there is normally never more than a single cycle of flowers above each one. I have never found an ovulate strobilus bearing staminate flowers.

2. *Abnormal.*—Several kinds of abnormal inflorescences have been observed which will be described in full in a subsequent paper. Only two kinds need concern us at present. In one the bracts do not form a series of collars but a continuous spiral. In other words the flowers are arranged in a spiral and not in cycles, as is usual (see fig. 1, Plate II). The turns of the spiral are about the same distance apart as are the cycles of the normal. Some strobili are partly spiral and partly cyclic. Sometimes the spiral elevation corresponding to the collars is broken up into a series of bracts. These abnormal strobili may be either staminate or ovulate but more frequently they are ovulate. They are by no means rare. I have observed them in every species with which I have worked. On some specimens no other kind of strobili was found, indicating that it is perhaps an hereditary character.

Although the anatomy of these strobili has not yet been studied certain inferences seem to be justified. It seems clear that they represent a return to an ancestral condition. The spiral arrangement is the prevailing one in all groups of Gymnosperms except the Gnetales and these abnormal strobili merely represent reversions to that primitive Gymnosperm condition. The cyclic arrangement in the strobili of Gnetum has then been recently acquired.

It should be pointed out further that these strobili bear a remarkable resemblance to the catkins of the lower Dicotyledons. There is a central axis bearing bracts arranged in a spiral and in the axils of these bracts are borne the flowers just as in the Amentales. Furthermore, evidence will be presented later (page 18) to show that the female flowers of Gnetum have an ovary and perianth similar to those of the Amentalean flowers. It is therefore evident that these abnormal strobili of Gnetum are remarkably similar to those of the
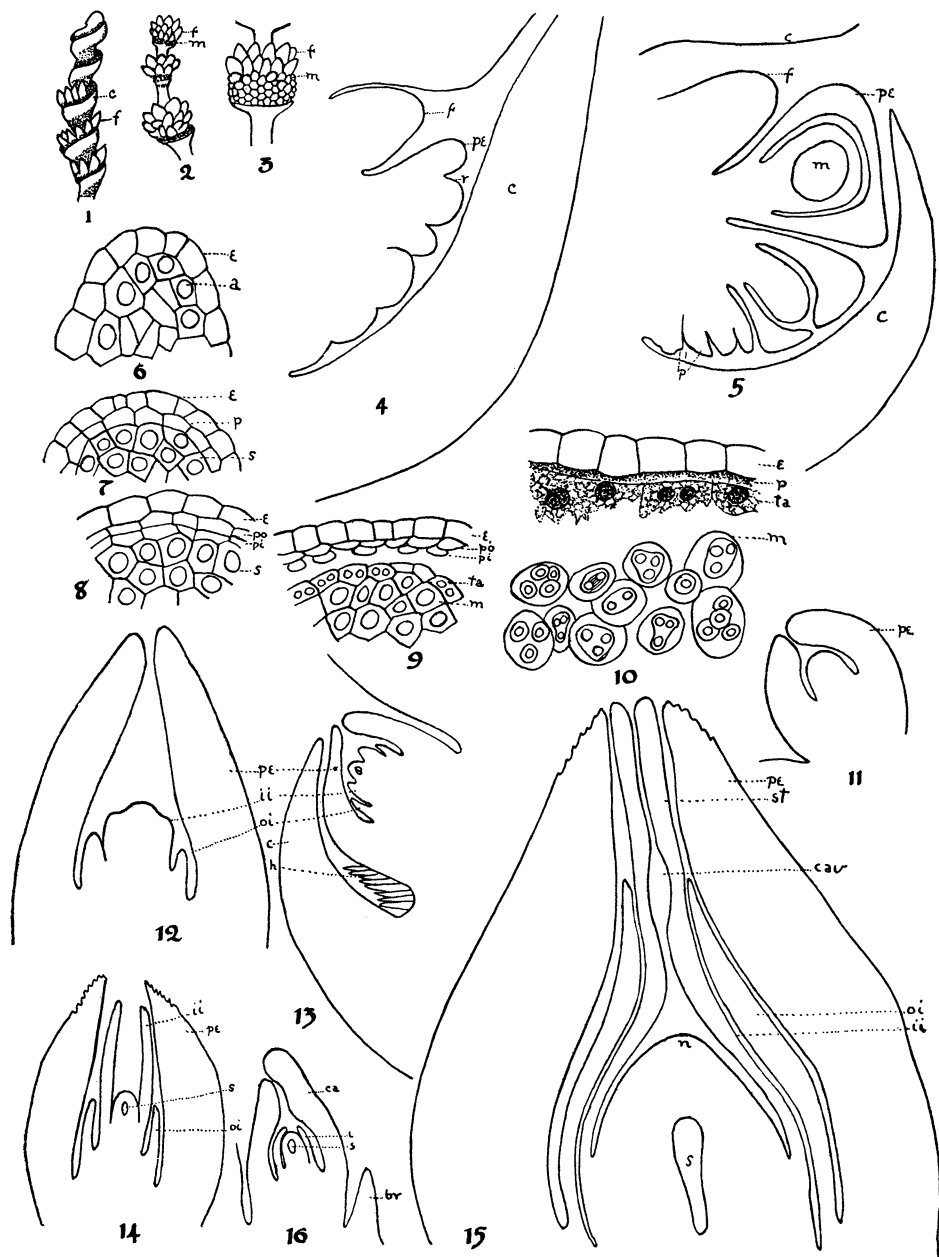
lowest Angiosperms.   Certainly if this type of Angiosperm was evolved from any forms at all closely related to the Gnetales it must have been while the latter bore strobili of this spiral type.

The second kind of abnormal strobilus is illustrated in figures 2 and 3.   It is a staminate one with many abortive ovulate flowers massed in several ranks in place of the usual single ring.   The strobilus represented in figure 2 is an old one from which the staminate flowers have fallen and on which the ovulate ones have grown considerably.   Figure 3 shows the young condition.   If one considers the male and female flowers as single stamens and ovules respectively, each provided with an envelope, the resemblance of each group with its collar to the Ranalean type of flower is striking.   Above is a large group of ovules; below these, numerous stamens arranged in a low spiral; lower still the bracts of the collar.   This is the arrangement in that type of Angiosperm flower which is considered by many botanists to be the primitive one.   Nevertheless it seems clear that this resemblance is only superficial.   Evidence will be presented later to show that both male and female flowers are themselves reduced from the bisporangiate condition.

3. *Anatomy.*—Only a preliminary study of the anatomy of the strobili, either normal or abnormal, has yet been made.   But, in addition to the presence of centripetal wood, it has revealed the occurrence of a type of vessel not seen elsewhere in Gnetum.   This is the familiar type of Ephedra.   It will be recalled that the ordinary vessel of Gnetum has a single terminal perforation like the vessels of most Angiosperms.   In fact the possession of this type of vessel is perhaps the most remarkable point of resemblance between Gnetum and Angiosperms.   But in the axis of the strobilus, an admittedly conservative region, in place of the single large perforation the vessels have a series of enlarged bordered pits from which the middle lamellae and tori have disappeared.   Now this is the ordinary type of vessel of Ephedra.   Therefore in this conservative region there persists a type of vessel characteristic of the most primitive member of the Gnetales—a type which has evidently been derived from the Coniferous tracheid (Thompson, 27).   The importance of these vessels in connection with the origin of the Angiosperm vessel is obvious. The difficulty arises, however, in that the primitive type of Angiosperm vessel appears to have scalariform end walls and not a single perforation.

5. Staminate Flower and Microsporangium

The young stamen is completely enclosed in an envelope known as the perianth which becomes ruptured at maturity. Stamen and envelope together constitute the staminate flower. The mature stamen resembles that of Angiosperms very closely except that it bears two sporangia instead of four. When it is remembered that the microsporophyll of no other group of Gymnosperms approaches this form, it seems that the resemblance to the Angiosperm stamen has not been sufficiently emphasized.

In the course of development the numerous flowers of a group arise in basipetal succession. Within a single collar one may find many stages. In the case represented in figure 4 the uppermost flower has already developed a perianth while the lowermost is a barely recognizable rudiment. In figure 5 the uppermost flower is in the mother-cell stage while the lowermost is just beginning to form a perianth. It is not possible to say that either perianth or stalk arises first because they arise together in a single rudiment from which the perianth becomes separated later by a circular depression (fig. 5 at the bottom). Then the perianth elongates and closes over the central rudiment of stalk and sporangia.

The archesporium consists of a hypodermal layer of cells which first becomes recognizable shortly after the perianth is differentiated (fig. 6). As usual it divides to form a primary parietal layer against the epidermis (fig. 7) and the primary sporogenous cells. The parietal layer divides again periclinally producing two layers of cells between the epidermis and tapetum (fig. 8). No further divisions take place in the wall cells. Indeed in some cases even the second layer is not formed and in other cases only a few of the primary wall cells appear to divide again. In all cases the cells of the inner layer become more or less rounded and separate from each other (fig. 9). Later the cells of the outer layer also become rounded at the ends. All the parietal cells then gradually degenerate leaving only a thin band of granular substance against the epidermis. Consequently there is nothing resembling the endothecium of Angiosperms. Indeed in the mature sporangium the spores are enclosed in a single layer of cells, the epidermis.

In the meantime the primary sporogenous cells have been dividing and have formed a considerable mass of tissue. Before the cells of

the parietal layers begin to separate from each other a cleft appears between them and the sporogenous tissue. The outermost layer of sporogenous cells then quickly takes on the characters of a tapetum, both cytoplasm and nuclei becoming very dense and two nuclei appearing in each cell (figs. 9 and 10). In this case at least there can be no doubt that the tapetum is derived from sporogenous tissue. By the time the tapetum is fully differentiated the sporogenous cells within it have reached the mother-cell stage. The latter cells become more or less rounded and separated and then follow the tetrad divisions which produce the pollen grains. These divisions are not simultaneous throughout the sporangium as in Angiosperms but within a single sac all stages may be found from the undivided mother-cell to the young spore (fig. 10). Both tetrad divisions are completed before walls are formed. The four pollen grains occupy only a small part of the space within the mother-cell. Before the tetrad divisions are completed the granular substance referred to is all that remains of the parietal cells, and the tapetum has also begun to disintegrate (fig. 10.) When the young pollen grains become free the whole sac consists of a single layered epidermis, the granular remains of tapetum and wall cells and the mass of young microspores.

At maturity the stalk of the stamen elongates, breaking through the perianth and projecting beyond the collar of the strobilus. Dehiscence occurs by means of a cleft at the top of each sporangium. The first stamens to protrude are, of course, the uppermost ones. These soon fall and their places are taken by those next below and so on until all the stamens within the collar have matured.

The period of time occupied by the events just described is much shorter than is usual in Gymnosperms. The whole course of development up to the shedding of the pollen takes place in a few weeks. The only Gymnosperm which approaches Gnetum in the rapidity of this development is Ephedra, another member of the Gnetales, in which according to Land (15) the staminate strobilus is first recognizable in December (in New Mexico) and the pollen is shed in April. Indeed the period of development is shorter than in even the spring-blooming Angiosperms in which as a rule the winter is passed in the mother-cell stage. The summer-blooming Angiosperms it seems are the only seed plants whose microsporangia develop so rapidly.

6. Ovulate Flower and Megasporangium

1. *Fertile*. (*a*) *Envelopes*.—The rudiment of the whole structure is first recognizable as an elevation just above a collar of the strobilus. From the periphery of this elevation a thick, many-layered envelope is differentiated which grows up and encloses the central protuberance (fig. 11). This envelope becomes the so-called perianth. After it has enclosed the central rudiment another and thinner envelope is differentiated in similar fashion. While the latter is still very small the depression appears which separates the third and innermost envelope from the nucellus (see fig. 12, in which this circular depression is barely recognizable). The three envelopes thus develop in acropetal succession. In all the species investigated by the writer this is the order of development. This statement is in accordance with the observations of the earlier investigators (Strasburger, 25) and contrary to those of Coulter (7) who states that the inner envelope appears before the middle one.

The inner envelope soon elongates enormously (figs. 13 and 14). It quickly extends beyond the middle envelope and later even beyond the outer one to form the so-called style. At pollination time its tip becomes flaring and lacerated and always holds a droplet of liquid in which the pollen grains lodge. Its inner structure undergoes a significant development. All the cells except the innermost layer remain small and elongated parallel to the axis of the style. The cells of the layer lining the cavity, however, become large and elongated at right angles to the axis (fig. 17). Their rounded ends project into the cavity. Their protoplasm and nuclei become very dense and deeply staining (fig. 18). In fact the whole layer appears to be nutritive. A little below the middle a considerable chamber is formed in which many of the pollen grains lodge, appearing to stick first against the projecting ends of the nutritive cells. Here the pollen grains germinate and the tubes grow down to the nucellus. The nutritive layer becomes disintegrated into a granular mass by the growth of the tubes. After the pollen grains germinate the passage above the chamber closes.

Not all the pollen grains germinate in the style. Indeed most of them germinate on the tip of the nucellus (fig. 35). Nevertheless the style is much more like that of Angiosperms than had been supposed. It not only catches the microspores but also serves to conduct

and nourish some of the pollen tubes, and has a particular tissue developed for this purpose. This is a fact which must be given much weight in regard to the morphology of the envelopes of the female flower. At first sight it offers strong support to the contention that the inner envelope is really an ovary homologous to that of Angiosperms.

Moreover the fact that the pollen grains may germinate at a distance from the nucellus is a point which in itself closely connects Gnetum with the Angiosperms and removes it from the Gymnosperms. As stated by Coulter and Chamberlain (8) "the chief contrast in the sporophyte is that in Gymnosperms pollination results in bringing the pollen grains in contact with the ovule while in Angiosperms the result of pollination places the pollen in contact with a receptive surface developed by the carpel." Whatever be the morphology of the inner envelope the essential point is that some of the pollen grains do not germinate on the nucellus as in Gymnosperms but at a distance from it as in Angiosperms. And by many botanists this is considered the chief contrast between the sporophyte of Gymnosperms and that of Angiosperms.

That part of the inner integument surrounding the nucellus remains thin and undifferentiated. At the maturity of the seed it forms a thin, more or less papery covering of the endosperm. The middle envelope becomes differentiated into two tissues, an inner hard, stony layer, and an outer thin papery one, closely investing the stony layer and containing many sharp spicular cells. This outer layer of the middle envelope is quite thick at the top of the endosperm. The outer envelope becomes very thick. Its tip is papillate. It contains many resin passages and star-shaped spicular cells. At the maturity of the seed it forms a thick, fleshy, edible layer of a bright red color.

Concerning the morphology of these envelopes there have been many opinions which may be summarized as follows: (1) All three are integuments resulting from the differentiation of the single integument of Gymnosperms (Strasburger, 25); (2) the two innermost ones are integuments and the outermost is a perianth (Beccari, 3) or something analogous to it (Coulter, 7); (3) the two innermost ones are integuments and the outermost is an ovary or something analogous to it (van Tieghem, 32); (4) the innermost one is a true Angiospermous ovary and the outer two perianth or something analogous to it (Lignier

and Tison, 17 and 18). These views seem to include all the possibilities and it will perhaps be difficult to choose between them until the anatomy is studied in a wider range of forms. Nevertheless the conditions described in the preceding pages have a bearing on the problem which should be pointed out.

In regard to the view that all three envelopes are integuments it is only necessary to remark that there is no evidence except their general appearance in favor of it. Such a view, moreover, fails to offer any explanation of the envelope of the male flower which is evidently of the same type.

The second view, namely that the two inner envelopes are true integuments and the outer a perianth or something analogous to it is the one most generally held at the present time and most convincingly stated by Coulter (7). This author pointed out that those coverings of the ripe seed which are derived from the two inner envelopes are the same as those derived from the single integument of Gymnosperms: an inner fleshy, a middle stony, and an outer fleshy. The inner fleshy layer of Gnetum is derived from the inner envelope and the other two from the middle envelope. Accordingly Coulter concluded that the inner envelope of Gnetum represents the inner part of the single integument of Gymnosperms and the middle envelope represents the remainder of this single integument. In other words the single integument of other Gymnosperms has become divided into two distinct integuments in Gnetum. Opposed to this view is the style-like character of the projecting portion of the inner envelope which strongly supports the view that it is really an ovary and not an integument. Further, the development and anatomy of this envelope in both Ephedra and Welwitschia indicate that it really consists of two fused members.

The third view (that there are two integuments and an ovary) is at first sight very attractive particularly when this flower is compared with that of one of the lower Angiosperms such as Peperomia. Figure 16 represents a section of a flower of Peperomia *sp.*, and if it is compared with a section of a Gnetum flower it is seen that the resemblance is very striking and that the carpel of Peperomia corresponds closely to the outer envelope of Gnetum. Moreover the development of the flower of Peperomia is almost a repetition of that of Gnetum (Johnson, 11). In form and position with respect to the remainder of the flower this envelope certainly resembles an ovary as

much as a true perianth. The objections to this view are as follows: (1) As previously shown the anatomy and development of the inner envelopes of other members of the Gnetales indicates that they are composed of two fused foliar members and that they are not integuments. (2) The outer envelope has not the functions of a carpel in collecting pollen and conducting pollen tubes. (3) The envelope of the staminate flower of Gnetum is obviously of the same type and certainly cannot be considered an ovary. The homology of these envelopes in male and female flowers is shown by their form, development and anatomy. (4) The similar envelope of the male flower of Welwitschia encloses the stamen cycle and therefore cannot be considered an ovary. These objections seem to constitute too great a body of evidence for the view to be longer tenable, despite the evident similarity in form between the outer envelope and a true ovary.

Finally there is the theory recently advanced by Lignier and Tison (17, 18) that the innermost envelope is a true ovary and that the two outer envelopes are in the nature of a perianth. This implies that the ovule is destitute of integuments. There appears to be much more evidence in favor of this view than any of the others partly because of the facts revealed in this article and partly because it accounts for the morphology of the similar envelopes of other Gnetalean flowers and indeed for their whole structure. It is clear that in any theory of the morphology of the envelopes in Gnetum, the structure of the female flower of Gnetum must be harmonized not only with that of the male flower but also with that of the flowers of the other genera of the Gnetales. The general argument as developed by Lignier and Tison, chiefly in connection with the flower of Welwitschia, will first be stated and then applied to Gnetum.

It is admitted by practically all investigators that the structure of the male flower of Welwitschia with its abortive ovule above the cycle of stamens indicates that the immediate ancestors of the genus bore hermaphrodite flowers arranged on the Angiosperm plan, and that the female flower has resulted from the suppression of the stamen cycle. Owing to the similarity of the flowers of other members of the Gnetales to those of Welwitschia it is evident that they too are reduced from a hermaphrodite condition. This conclusion is independently confirmed by the discovery (18) of abnormal flowers of *Gnetum scandens* bearing stamens within the second envelope. With this general arrangement in mind we may now consider the morphology of the individual parts.

That which is called the integument of the Welwitschia ovule is, according to the theory, really an ovary. Lignier and Tison justly lay much emphasis on the style-like character of its projecting tip. Furthermore we have already called attention to the fact that its development and anatomy both in Ephedra and Welwitschia indicate that it is an ovary of two fused members. The stamen cycle of Welwitschia is fused at the base and divided above into six parts. But its anatomy shows that there are really two members (as in the other cycles) which branch above. This is assumed to be the ancestral condition for the group. The perianth consists of two pairs of bracts. In Welwitschia the first pair are connately used and the second pair represented by bractlets. In Ephedra both pairs are connate. The ancestral flower of the Gnetales therefore consisted of an ovary of two fused members, two stamens and two pairs of decussate bracts.

It must be admitted that one finds difficulty in imagining how the staminate flower of Gnetum can have been reduced from such a type. This flower consists of a single stamen surrounded by a single envelope. It must be assumed that the ovary, one stamen, and one pair of perianth bracts have disappeared and that the other stamen has taken a position at the top of the axis. Although this seems a big assumption to make I believe it is justified particularly in view of the certain abnormalities which will be described in full elsewhere. They consist in brief of male flowers which had grown out into axes of considerable length and complexity.

We are now in a position to apply the general conception of the Gnetalean flower to the structures in the female flower of Gnetum. It is plain that the inner envelope of Gnetum is even more like an ovary than that of Welwitschia for it not only resembles an ovary in form and anatomy but it also bears a style on which the pollen is caught and in which some of it germinates. Accordingly we conclude that the first envelope is really an ovary whether or not it is the homologue of the Angiosperm ovary. The second and third envelopes then represent fused bracts which may or may not constitute a true perianth. The stamen cycle has disappeared. The whole view receives strong support from the discovery of abnormal flowers bearing stamens within the second envelope.

This body of evidence seems to demonstrate that we have at last obtained the proper interpretation of the envelopes of Gnetum.

Not only does it satisfy all the conditions in Gnetum but it also harmonizes these conditions with those in both the other genera of Gnetales.

The conclusions which have just been reached have a profound significance in connection with the relationship between Angiosperms and Gnetales. If the reduction hypothesis is correct the ancestral flower of the Gnetales had all the parts of the Angiosperm type arranged in the same manner as that type. We may now enquire whether those individual parts correspond exactly.

It is clear that what we have called an ovary in Gnetum is in all essential respects the same as that of Angiosperms. It is a sac derived from foliar members enclosing an ovule and bearing a special structure on which the pollen is received and in which some of it germinates. The real question appears to be whether Gnetum is a true Angiosperm. For all practical purposes it is Angiospermous.

The ovule is single and orthotropous, rising from the base of the ovarian cavity. These are the conditions in some of those Angiosperms which are classified on the basis of other characters at the bottom of the phylum—the Amentales. Some of the latter, *e. g.*, Salicaceae, have more than one ovule in each ovary but even in Gnetum I have seen in abnormal instances two ovules developing in an ovary. The chief difference as far as the ovule is concerned is the absence of integuments in Gnetum (according to our interpretation). But the ovules of certain Angiosperms also lack integuments and it is not strange that the integuments should disappear in flowers reduced in so many other ways as are those of Gnetum.

In most Angiosperms the ovules are attached to the side of the sporangial cavity and are therefore said to be foliar. Those which rise from the bottom of the cavity are obviously attached to an axis and are said to be cauline. The old view that ovules are modifications of some part of a leaf implied that the foliar condition is primitive and that the cauline one has been derived from it. This conclusion is opposed by the evidence derived from the distribution of the two types, the cauline condition being found chiefly among primitive forms and the foliar among the higher families. The mistake lies in the original assumption that ovules are modifications of some part of a leaf. Ovules are ancient members of the plant body and hold no necessary relation to either leaf or stem. As stated by Coulter and Chamberlain (8) they have a history which probably antedates that of

both leaf and stem. There is no reason why they cannot occur on the base as well as on the sides of the sporangial cavity. Therefore the obviously cauline character of the ovules of Gnetum cannot be used as an argument against its being related to the type from which Angiosperms were derived. Indeed it rather supports that view because cauline ovules are found chiefly among primitive Angiosperms.

The stamen of Gnetum has already been compared with that of Angiosperms.

The perianth of the Gnetales according to our interpretation consists of two pairs of connate bracts. Among the Amentales the perianth consists of similar small colorless bracts which are either distinct (*e. g.*, Myricaceae), or fused (*e. g.*, Juglandaceae, Betulaceae). Therefore it appears that there is more than mere analogy between the perianth of primitive Angiosperms and that which we have called perianth in Gnetum.

We conclude that in regard to every part the flower of Gnetum closely resembles that of primitive Angiosperms. And we have already seen that the arrangement of the flowers themselves particularly in the abnormal spiral strobili is just the same as in those primitive Angiosperms. The flower of Gnetum is, therefore, not far removed from the type from which that of Angiosperms was derived.

This view implies that the Amentalean flower is the most primitive type found in Angiosperms. Another admittedly primitive type is that of the Ranales with numerous carpels, stamens and floral leaves all arranged in spirals. But if the Gnetalean derivation of the Angiosperms is the correct one this type must be considered more specialized than the Amentalean one. In this connection it is interesting to recall the structure of those abnormal strobili previously referred to in which there is a mass of ovulate flowers above the usual set of staminate ones. If the Gnetalean flowers be considered as simple ovaries and stamens and not as reduced from a hermaphrodite condition, the resemblance of these abnormal strobili to the Ranalean flower is marked. But all the evidence indicates that they are really reduced and therefore we must conclude that this resemblance is only superficial.

(*b*) *Nucellus and Archesporium.*—As stated by Strasburger (25) the archesporium always consists of two or more hypodermal cells. I have examined an abundance of material in all the early stages and have always found that as soon as an archesporium is recognizable

it consists of at least two cells and usually of three or four (fig. 19). It makes its appearance at about the time that the inner envelope (ovary) becomes differentiated. The succeeding events take place in the usual manner. The archesporial cells by periclinal walls cut off the primary wall cells (fig. 20) which, together with the epidermis develop a large mass of tissue above the sporogenous cells. The cells of the upper part of the nucellus become densely charged with cytoplasm which contains many starch grains. No definite pollen chamber is formed though the tip of the nucellus usually becomes disorganized by the ingrowing pollen tubes. That part of the nucellus at the base of the embryo-sac develops a peculiar nutritive pavement tissue which according to Coulter (7) was mistaken by Lotsy (19) for tissue within the sac. But it will appear later that Lotsy was correct in stating that there may be cellular tissue in the base of the sac when there are only free nuclei at the top. It will also appear, however, that Lotsy was mistaken in stating that this cellular tissue was present before the pollen tube enters the sac. It is in reality endosperm tissue which develops after the entrance of the pollen tube and is quite distinct from the pavement tissue outside the sac.

The whole ovule undergoes a rapid development while the pollen tubes are growing through the nucellus. Soon after pollination time (indicated by the flaring style with its droplet of liquid) certain ovules are seen to be growing rapidly while others remain the same size. One naturally concludes that the growing ovules have been fertilized and that the endosperm is forming. On sectioning such ovules, however, one invariably finds that the pollen tubes have not yet reached the sac. Not until the ovules are considerably larger than those which failed to be pollinated does one find pollen tubes in contact with the embryo-sac. It seems, therefore, that the presence of pollen tubes in the nucellus stimulates the latter to further development.

(c) *Sporogenous Tissue.*—According to Strasburger (25) the primary sporogenous cells divide to form the mother cells, a very unusual behavior in Gymnosperms. My sections show clearly that this is not the case but that the primary sporogenous cells as usual function directly as mother cells. There are accordingly from two to four of the latter. Figures 20 and 21 show these cells in longitudinal section and figure 22 in transverse section. The mother-cells divide to form the megaspores while the ovule is still very young—in

fact while there are only three or four rows of cells between the spo-
rogenous tissue and the epidermis. Figure 23 represents a case in
which one mother cell is dividing while the one beside it remains
undivided. In figure 24 the two deeper lying cells have divided once
and the more superficial cell has remained undivided. In figure 25
are represented the products of two mother cells. At the left is a
linear row of three cells showing that one daughter cell only has
divided to form megaspores. At the right are three similar cells the
lowermost of which (megaspore) has divided and produced a four
nucleated embryo-sac. In general the outer daughter cell of the
first division fails to divide again. So far as I have observed it is
always the deepest megaspore which functions. Invariably two and
frequently three mother cells produce megaspores. In case one does
not divide it is always the outermost. Usually a megaspore from
each mother cell which divides develops into an embryo-sac. For
this reason there are almost always more than one embryo-sac in a
mature ovule, usually a large central one and one or two smaller ones
at its outer end.

2. *Abortive Megasporangia.*—It will be recalled that in every
staminate strobilus there is a ring of abortive ovulate flowers above
each set of staminate flowers. It has been reported (Lotsy, 19) that
these flowers occasionally produce fruit and this statement I can
confirm. It is my observation that while the great majority of
staminate trees never produce fruit from these ovules an occasional
tree will produce large numbers. If one finds fruit developing in a
staminate strobilus one is almost certain to find many other examples
on the same tree. It seems therefore to be a definite tendency,
probably inherited, in certain trees and not an occasional event on
any tree. Of course the axis of the strobilus becomes much stronger
and thicker than it otherwise would. Frequently the axis is unable
to develop strength enough to nourish these abnormal fruits for often
I have seen staminate strobili with several half developed fruits be-
coming yellow and sickly. Though very few of the ovules in staminate
strobili produce fruit a good many of them develop embryo-sacs
which appear to be of the usual type. Figure 27 shows a transverse
section of an abortive ovule with the embryo-sac in the free nucleate
condition.

It is always stated that these flowers differ from the typical flowers
of the ovulate inflorescence in that they have only two envelopes.

The relationships of these envelopes to those of the typical flower were not understood. I have observed that every abortive ovulate flower at a certain stage in its development possesses a rudiment of a third envelope between the two well-developed ones (see fig. 26). It seems evident, therefore, that the envelopes present are the inner one (ovary) and the outer which we call perianth and that the middle envelope is represented only by the vestige present during development. The invariable presence of the middle envelope although in a rudimentary condition, together with the frequent presence of a typical embryo-sac and the rare development of fruit, shows that these flowers are quite homologous with the functional ovulate flowers. And this homology suggests that the immediate ancestors of Gnetum had bisporangiate strobili. In view of the conclusion that the flowers themselves were originally bisporangiate (page 18) and have been reduced to the monosporangiate condition, it is difficult to understand why the bisporangiate strobili should have been developed and then reduced.

7. Male Gametophyte

Until the publication of Pearson's (22) incomplete account of conditions in G. africanum our only knowledge of the male gametophyte of the genus was the statement of Lotsy (19) that the tube nucleus and two male cells of the ordinary type are in the pollen tube just before fertilization. The writer has been able to observe almost all stages in the development of the male gametophyte in G. gnemon, G. latifolium and G. sp. 33 and several stages in other species. The different species are quite alike in all essential points.

The young microspore on being freed from the cavity of the mother cell has a very thin wall in which only one layer can be distinguished. Later a second layer appears and the typical exine and intine are present. The exine becomes covered with very small protuberances.

The nucleus of the young microspore is large and rather dense and has a prominent nucleolus. This nucleus divides very soon after the microspore is freed. The first division does not give rise to a prothallial cell as in so many Gymnosperms but to a tube nucleus and generative cell (see fig. 29). No prothallial cells are formed. In this important respect, therefore, Gnetum has completely departed from the Gymnosperm condition and has arrived at the Angiosperm

condition. The tube nucleus is slightly larger than the generative nucleus but is much less dense and has a very distinct nucleolus. In most preparations it is impossible to distinguish the cytoplasm of the generative cell from the general cytoplasm of the spore. But in well-stained sections the generative cytoplasm is seen as a narrow lighter band surrounding the generative nucleus, the whole cell being only slightly larger than the tube nucleus. The generative nucleus is very dense and deeply staining. Very soon the generative cell divides to form a small stalk cell and a body cell (fig. 30). In this condition the pollen grain is shed, and consequently at pollination time the gametophyte consists of a tube nucleus in the general cytoplasm, a body cell and a stalk cell. The tube nucleus is distinguishable by its greater size, lighter appearance and conspicuous nucleolus. It is very often difficult to see that the body nucleus and stalk nucleus are each surrounded by its own cytoplasm. The body nucleus is smaller than the tube nucleus and very dense. The stalk nucleus is very small and dense. Frequently the stalk and body nuclei appear to be in the same cytoplasm (fig. 31).

It is particularly easy to watch the growth of the pollen tube because as previously stated the pollen grains frequently germinate in the style. The exine is thrown off as usual and the cavity of the style in later stages frequently contains many of these outer coats. The intine grows out into a tube at the point nearest the tube nucleus. Figure 32 represents a tube which has branched though far from the nucellus. The tube nucleus with its conspicuous nucleolus is next to the branched end. Back in the pollen grain proper are the body and stalk cells, each with its own cytoplasm. As the tube grows the tube nucleus and body cell pass out into it (fig. 33). The stalk nucleus seems invariably to stay behind in the old grain. There it remains for a long time until finally it degenerates. Figure 35 shows the tip of a nucellus with many pollen grains which have germinated and sent tubes far down towards the embryo-sac. In all cases the tube nucleus and body cell have passed down the tube but the stalk nucleus has remained in the old pollen grain. Some of them have begun to disintegrate. Old germinated pollen grains, whether in the style or on the nucellus usually show remains of this stalk nucleus. So far as I am aware this behavior occurs in no other Gymnosperm. It seems to be a step towards the complete elimination of the stalk cell.

The body cell never divides until it is in the pollen tube. Figure

33 shows a tube in which the cell is just entering the nucellus. The tube nucleus is down at the tip of the tube. It will be observed that the body cell is undivided. Unfortunately I have never seen its division, but by the time it is half way through the nucellus it always contains two nuclei, consequently the division must occur in the upper part of the nucellus. It is usually impossible to see in my preparations that there are two distinct male cells. Generally I can see only two nuclei lying in a common cytoplasm (fig. 34). In favorable sections, however, one can see that this cytoplasm is divided into two by a delicate membrane (fig. 40). It may be that in all cases there are two distinct male cells but this I am not prepared to say. In the lower part of the nucellus the tube nucleus and male cells lie side by side, with the former in advance. Against the sac the tube nucleus is found at the side of or behind the male cells. The tube nucleus is always larger than the other nuclei and has a prominent nucleolus. There is no difference in size between the male nuclei or cells.

The most notable points in this gametophyte are (1) the absence of prothallial cells, (2) the germination of the pollen grains in the style as well as on the nucellus (3) the retention of the stalk cell in the pollen grain and (4) the division of the body cell in the nucellus. The first three points bring the male gametophyte of *Gnetum* very close to that of Angiosperms.

8. FEMALE GAMETOPHYTE

As previously stated two or three megaspores usually develop into embryo-sacs. Each of these functioning megaspores is descended from a different mother cell. Figure 25 shows a four-nucleate embryo-sac and alongside it a megaspore derived from another mother cell. At the end of the sac and of the undivided megaspore are the sister megaspores which will not function.

The subsequent divisions in the gametophyte take place in the usual Gymnospermic fashion. A vacuole soon appears in the center of the developing sac and the protoplasm and nuclei become confined to a parietal layer. Figures 36 and 37 illustrate the conditions found in the young sac, the latter figure also showing the typical appearance of two sacs in one nucellus at this stage. Very often the vacuole appears first at the upper end of the sac (fig. 38) and gradually extends downward. In any case the upper end always becomes larger
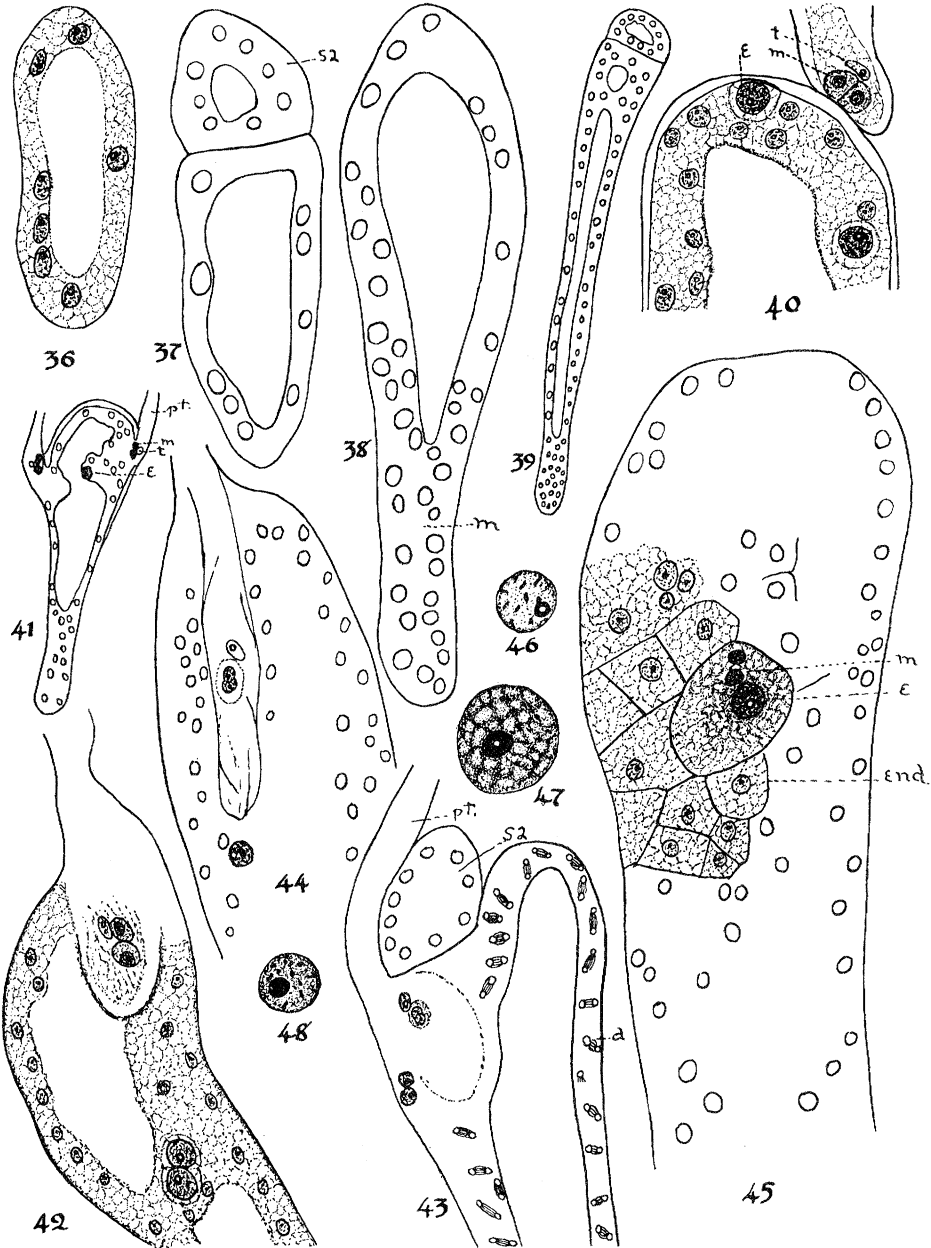
than the lower, the whole then taking on the form of an inverted flask. Usually the vacuole does not extend completely to the bottom of the sac, this end being occupied by a mass of protoplasm and nuclei (figs. 38, 39). This fact is important in connection with the formation of the endosperm. Figure 41 shows the appearance of the mature sac in *G. gnemon* and figure 39 that of *G. sp. 33*. Each is seen to be shaped like an inverted flask with a mass of protoplasm and nuclei at the lower end and a thin layer of protoplasm containing a single row of nuclei along the sides. Occasionally strands of protoplasm containing nuclei stretch across the upper end. In *Gnetum gnemon* the neck of the flask is not nearly so long as in *G. sp. 33* and other species.

The nuclei have almost the same appearance throughout the development of the sac, but gradually get slightly larger. Each one contains very little chromatin matter and a large conspicuous nucleolus in the form of a hollow ball. The cavity at the center of the nucleolus is usually very plain. The divisions in the sac are simultaneous. The number of nuclei finally produced in *G. gnemon* is approximately 256, which is the usual number found in Gymnosperms before wall formation takes place. It is evident therefore that in this species the development of the gametophyte follows the typical Gymnosperm method in all stages prior to the formation of cells. At this point the similarity ends. In *G. sp. 33* and *G. moluccense* the total number of nuclei is approximately double the usual Gymnospermic number or 512. In other words another division of each nucleus has taken place.

As stated by Thomson (30) the megaspore membrane (wall of the embryo-sac) is very thin. In all the species examined I could distinguish only a single thin homogeneous membrane which almost disappears towards the top of the sac. In *G. sp. 33* this membrane becomes considerably thicker during the early stages of endosperm formation.

After the pollen tube comes in contact with the embryo-sac a very important development takes place. One or more of the nuclei at the upper end of the sac become differentiated from the others and definitely recognizable as egg nuclei. They can easily be distinguished from the remaining nuclei by their larger size, greater affinity for stains, very dense structure, and inconspicuous nucleolus. The other gametophytic nuclei always have a loose structure and very conspicuous nucleolus. It is not always possible to satisfy oneself that the egg nucleus has its own cytoplasm and limiting membrane.

I believe that the egg cytoplasm and membrane are always differentiated and it may be that in cases where they cannot be distinguished the egg nuclei have just been organized and that the cytoplasm and membrane will be differentiated later.   In any case the nuclei themselves are always plainly differentiated.   Usually two such eggs are present, often only one, and sometimes three.   Figure 40 shows the upper end of a sac in which two eggs have been differentiated.   The pollen tube is seen pressed against the sac.   Figure 41 gives the appearance of the whole sac at this time.   The eggs are also shown in the sacs in figures 42, 43 and 44.   Figure 47 represents an egg nucleus alone.

Apparently these eggs make their appearance only under the stimulus of the presence of the pollen tube against the sac.   It seems that the pollen tube remains pressed against the sac for a long time before bursting in, because it is found in this position more commonly than in any other in ovules of about this age.   One can frequently find sacs in contact with pollen tubes although no eggs have yet been differentiated.   But before the pollen tube enters the sac, the eggs are always visible.   Therefore one concludes that the eggs become differentiated only when the pollen tubes are in contact with the sac.

The position of the nuclei which become transformed into eggs bears no definite relation to that of the pollen tube.   Though they are always in the upper part of the sac they may be either directly under the pollen tube or at the opposite side of the sac and are often at some distance from the end.

The fact that Gnetum forms definite eggs is one of the outstanding results of this investigation.   It has always been supposed that any of the free nuclei in the gametophyte might be fertilized (Lotsy, 19). "It is in this stage that fertilization occurs, for the free nuclei are potential egg nuclei, although a group at the antipodal end of the sac may be as distinctly vegetative as are the antipodal cells of Angiosperms" (Coulter and Chamberlain 8).   If this is true then of course all the nuclei in an Angiosperm gametophyte are in the same sense potential egg nuclei.   In fact the differentiation of special nuclei to serve as eggs makes the resemblance of the female gametophyte of Gnetum to that of Angiosperms still more striking.   Particularly does it strengthen the resemblance, already frequently pointed out, to the irregular sacs such as those of Peperomia (Campbell, 6, Johnson, 10, 11), Gunnera (Schnegg, 23), etc., in which many free nuclei are

found and in which no polarity is evident. Many such sacs have been described in recent years and it is by no means proven that they are specialized and not primitive as was originally contended by Campbell but disputed by Johnson. When the development of the endosperm is described (page 34) it will be seen that there is a still further resemblance. It seems particularly significant that Peperomia should also resemble Gnetum in many points of flower structure and that it should be classified by universal consent among the very lowest of the Angiosperms.

Whether or not the female gametophyte of Gnetum represents the condition from which the Angiosperm gametophyte was really derived, it seems to have a bearing on the morphological nature of the cells within the Angiosperm embryo-sac. Many attempts have been made to relate the egg and synergids to the archegonium of lower plants. It has been urged at different times (1) that they represent the egg and canal cells of a single archegonium, (2) that all three represent archegonia, and (3) that only the egg represents an archegonium while the synergids represent the upper part of the prothallus (Berridge and Sanday 4). The conditions in Gnetum show that there is no need to relate the eggs in any way to archegonia but merely to consider them as eggs produced by a gametophyte which cannot form archegonia. The absence of cellular tissue prevents the formation of archegonia and hence free nuclei organize as eggs. If this is true for Gnetum it is still more evidently true for Angiosperms even though there be no genetic connection between them.

The conditions in the female gametophyte may be summarized as follows: the whole sac is shaped like an inverted flask; at the mouth of the flask is a considerable mass of cytoplasm and free nuclei; along the sides of the neck a narrow band of cytoplasm with one row of nuclei; in the body a broader band of cytoplasm with one or more rows of nuclei; in variable positions one or more eggs. The inferences to be drawn from these conditions are: (1) The early development is Gymnospermic, (2) the later development and mature condition is even more suggestive of Angiosperms, particularly of the irregular forms, than had been supposed, (3) it is impossible to relate any structures in this or the Angiosperm gametophyte with the archegonia of lower forms.

9. FERTILIZATION

The entrance of the pollen tube into the embryo-sac will be described under the head of fertilization, although in some species at least several important events intervene between the entrance of the tube and the essential act of fertilization, the fusion of male and female nuclei.

The point of contact between pollen tube and embryo-sac varies in position but it is usually at the side of the expanded part of the sac. The tube nucleus takes up a position at the side of or behind the male cells. The contents of the pollen tube are discharged into the sac·in the usual way. Both male cells, the tube nucleus, and a certain amount of cytoplasm pass in. The contents of the sac draw back slightly though not pushed back by any wall or mass of protoplasm. The male cells then make their way to one of the eggs, often traversing a considerable space in doing so, and leaving the tube nucleus behind. Figure 41 shows the general arrangement of the sac and tubes. One tube is entering at the left and another further up at the right. The egg is visible at the inner edge of a mass of cytoplasm. Figure 42 shows part of a sac under greater magnification. The male cells and tube nucleus are just entering and the two eggs are to be seen further down in the sac. Figure 43, introduced for another purpose, represents about the same stage. In the case represented in figure 44 the male cells have penetrated far into the sac towards the egg. The clear space ahead bounded by a partly collapsed membrane is typical. The subsequent events depend upon the species.

In *G. sp. 33* a considerable time elapses before the actual fusion of the nuclei, and in the meantime important events take place in endosperm formation. The egg and male nuclei are then found in a definite chamber partly or wholly surrounded by cells of the endosperm formed in a way to be described later (page 34). Figure 45 shows a large cell containing the egg nucleus and the two male nuclei, the whole cell surrounded by the cells of the endosperm. Further away are free nuclei. In figure 49 the cells are seen to have formed around the end of the pollen tube which contains the tube nucleus. A large cell in the center contains the egg and male nucleus. Down in the neck of the flask are the endosperm cells which extend right to the bottom of the flask. Figure 50 shows the two distinct male cells in contact with the egg, all three enclosed in a definite chamber.
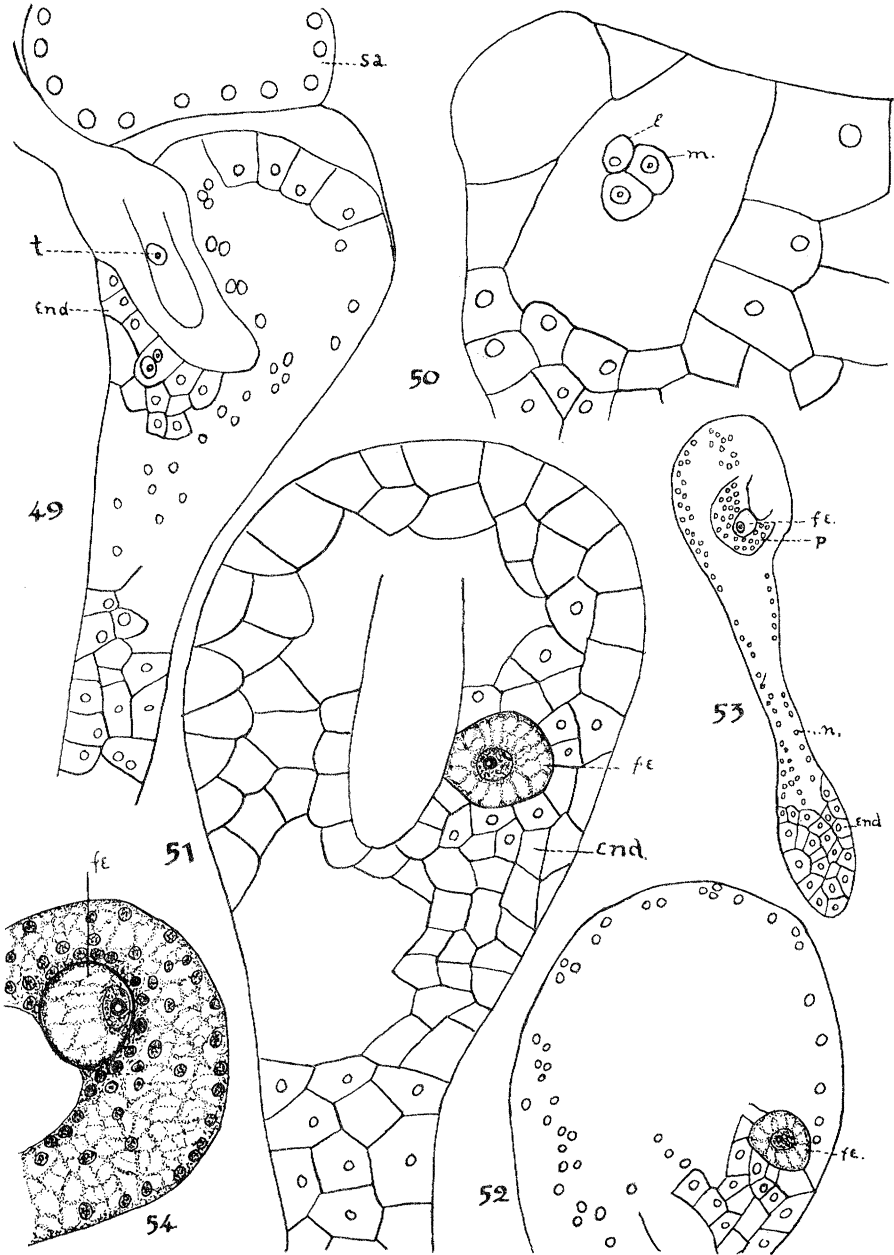
The conspicuous fusion nucleus is relatively very large and is situated in the center of a large cell.   It is quite impossible to distinguish the male and female chromosomes.   In view of the independence of the maternal and paternal chromosomes until after the first division in many recently described cases, I have examined this phase of the life history very carefully but have always found that the two nuclear masses become quite indistinguishable and that later a single large nucleolus is formed.   The cell containing the fusion nucleus is usually in contact with the old pollen tube.   Sometimes the embryo-sac in which it is found is nearly filled with cellular endosperm; sometimes it contains many free nuclei.

In *G. gnemon* the sexual cells are not surrounded by a cellular endosperm.   The cell containing them is, however, surrounded by a more or less definite mass of cytoplasm with many free nuclei.   It is usually found either against the end of the pollen tube or the wall of the embryo-sac.   I have not seen the actual fusion of sexual nuclei in *G. gnemon* and consequently cannot state exactly when it takes place with respect to the formation of this protoplasmic mass.   Figure 53 represents the whole sac at this time and figure 54 the fertilized egg surrounded by the body of protoplasm and nuclei.

The possibility of the occurrence of typical "double fertilization" will be discussed in connection with the endosperm.   But I wish to recall at this point Lotsy's (19) statement that both male cells function in the production of embryos, or, in other words, that twice the number of embryos are formed as there are entering pollen tubes.   While this may be the case occasionally in *G. gnemon*, my sections show that in the great majority of cases only one functions as is usual.   In most cases one fertilized egg only is found in a sac.   Lotsy was probably led into error by the number of suspensors which are produced by a fertilized egg (page 44).   The only evidence I have found in favor of Lotsy's statement is one case in which two fertilized eggs were present, although only one tube had apparently entered the sac.   Whatever be the conditions in *G. gnemon*, certainly only one male cell usually functions in other species.   I have often seen both male nuclei in a compartment with an egg and obviously the one which would not fertilize this egg could not fertilize any other.

10. Endosperm

As soon as the contents of a pollen tube enter the embryo-sac all the nuclei within the sac except the egg nuclei begin to divide. The divisions take place simultaneously throughout the sac. The similarity of the stages in all the dividing nuclei is remarkable. Figure 43 represents such a sac with all its nuclei in the same stage of division. The male nuclei can be seen entering at the left beneath the small second embryo-sac. Further down against the wall are two eggs. The divisions are repeated once or twice and with great rapidity. The nuclei which had all been large and loose in structure become greatly reduced in size and very dense as well as very numerous. In *G. gnemon* the subsequent events differ from those in other species and will be described separately.

(*a*) *G. gnemon.*—In this species although the parietal layer of protoplasm becomes thicker and encroaches on the vacuole, nevertheless the latter remains for a long time. In the thick band of protoplasm one finds a large number of small, deeply staining nuclei. The number is particularly large in the mass of protoplasm at the bottom of the sac and in that surrounding the egg. Then at the base of the sac walls appear in such a way as to form compartments. Each of the latter contains a mass of protoplasm and several (up to 10) nuclei. The walls are formed first at the extreme base of the sac and then gradually higher and higher up in the parietal layer of protoplasm. The result is a mass of cellular endosperm in the form of a shallow cup at the bottom and the parietal band of free-nucleated protoplasm above. In each cell are several nuclei. Figure 55 represents a sac in which walls are just beginning to form at the bottom, and figure 56, a later stage in which walls are forming higher up in the parietal protoplasm. It will be observed that each compartment is multinucleate.

The next step is the fusion of all the nuclei in each compartment into a single mass. The fusion takes place first in the lowermost compartments and then progressively in the higher ones. Consequently in certain sacs various stages in the process can be observed. Figure 57 represents a section of the base of a sac in which fusion has taken place in the lowermost compartments but not in the highest. As this section is tangential the vacuole in the center of the sac does not appear. Figure 58 shows a later stage in which fusion has
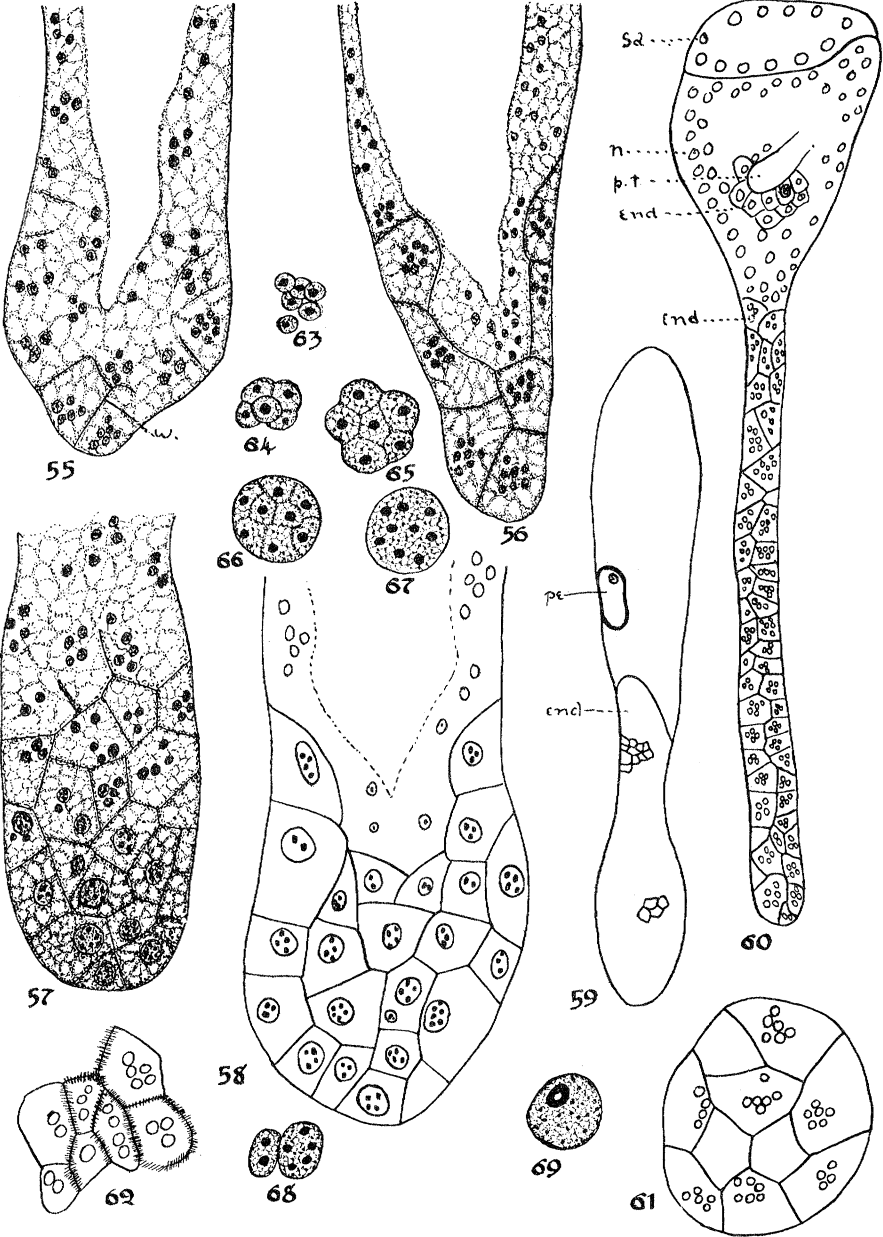
occurred in all the compartments.   There results a mass of uninucleate cells with a more or less deep depression in the center (in the position of the vacuole).   No more compartments are formed in this way. Indeed, even while fusion is occurring in the uppermost compartments division is taking place in the lowermost.   Figure 53 shows a whole sac at this stage.   At the bottom is the mass of uninucleate cells; above is the parietal layer of protoplasm with its free nuclei; and in the upper expanded part of the sac is the fertilized egg surrounded by a dense mass of protoplasm and nuclei.

Some stages in the fusion of the nuclei are represented in figures 63 to 69.   Figure 63 shows the small nuclei grouped together.   In figure 64 the membrane of the central nucleus is intact while those separating the other nuclei are disappearing and are represented by dotted lines.   In figure 65 the outlines of the individual nuclei are still plain though the membranes between them are breaking down. In figure 66 the whole mass has lost its scalloped edge and has assumed more of the appearance of a single nucleus, although the boundary of each constituent nucleus is still visible.   In figure 67 the individual nuclei are no longer distinguishable, although the nucleoli derived from them remain distinct.   The presence of a large number of nucleoli in these fusion nuclei is characteristic for a considerable time.   Sometimes all the nuclei in a compartment do not fuse into a single mass at once, but first form two or more masses which later fuse to one (fig. 68).   Sometimes one small nucleus may remain distinct until all the others have fused and then be absorbed into the general mass.   After a number of divisions of the fusion nucleus the numerous nucleoli (each derived from a constituent nucleus) disappear and a single larger one with the characteristic cavity in the center is found (see fig. 69).   The number of nuclei which fuse appears to vary widely.   I have never observed fewer than three nor more than ten.

The further growth of the endosperm takes place entirely in the cellular mass at the bottom.   All the cells divide but the growth is most rapid at the extreme lower edge of the sac.   Both cytoplasm and nuclei are much denser here than in the cells at the top of the endosperm (fig. 57).   Owing to its more rapid growth the lower part of the endosperm at first becomes wider than the upper part.   Gradually the tissue grows up the narrow part of the flask but always leaves the expanded part empty.   The cavity can be seen with the naked
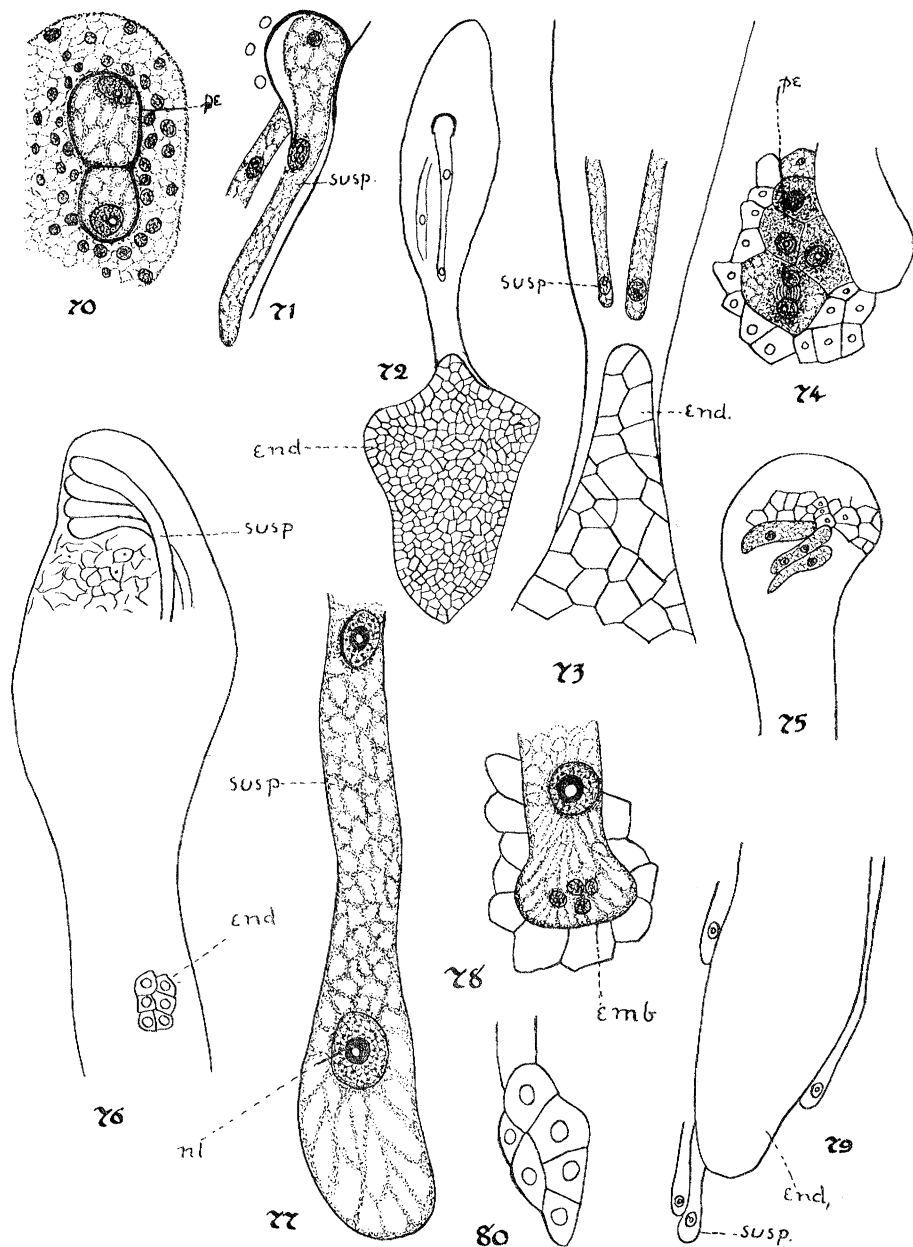
eye in young fruits. Most of the growth takes place downwardly
aned laterally. Figure 59 shows a whole sac with young endosperm
at the bottom and the fertilized egg at the side. The endosperm
never grows much higher than this. Figure 72 shows a later stage
and one which is often found.

It is plain from this description that Lotsy's statement is correct
that at a certain time there is a cellular mass at the bottom of the
sac while there are only free nuclei above. Coulter (7) thought
that Lotsy had mistaken the peculiar pavement tissue in the nucellus
(page 21) for tissue within the sac. Lotsy was in error, however, in
stating that this cellular tissue is present before the pollen tube enters.
As we have seen it does not develop until the male cells are within
the sac, though I am not prepared to say that compartments are not
present before the actual fusion of egg and sperm has taken place.
Not having seen the actual fertilization I cannot say with certainty
at what stage of endosperm formation it takes place in this species.
But I have frequently observed young fertilized eggs in sacs in which
compartments had already formed. It seems that the formation of
compartments and fusion of endosperm nuclei go on concurrently
with fertilization. Lotsy thought that the female gametophyte of
G. gnemon represented an intermediate condition between that of
Gymnosperms and Angiosperms in that at fertilization time the
endosperm was partly cellular and partly free-nucleated. Although
this gametophyte does represent an intermediate condition, it is
much more like that of Angiosperms than Lotsy believed, because
when the pollen tube enters it contains only free nuclei and eggs.

The free nuclei in the upper part of the sac above the region of
cell formation take no part in the production of endosperm. Except
in the immediate vicinity of the fertilized egg they all disintegrate
more or less rapidly. Those which surround the fertilized egg in-
crease in number for a time and form a dense, deeply staining mass,
which is well defined (fig. 53). I have occasionally observed the
formation of compartments here similar to those at the bottom. The
function of these nuclei and of the protoplasm in which they lie,
appears to be the nourishment of the fertilized egg. But sooner or
later these also degenerate leaving the fertilized egg alone in the
cavity of the embryo-sac some distance above the endosperm.

(*b*) *G. sp. 33.*—In this species and similar ones endosperm formation
takes place in a somewhat different manner. As soon as the pollen

tube enters, the same rapid divisions occur throughout the sac and result in the production of a large number of small nuclei. But in these forms wall formation also takes place in the expanded part of the sac in the vicinity of the egg. It is particularly easy to observe it here. The spindle fibers of the last few divisions remain and consequently each nucleus is connected by fibers with several others. The walls are formed across these groups of fibers in the usual way. Not all the groups, however, are involved and consequently compartments are formed containing several nuclei (see fig. 62). The fibers not involved in wall formation disappear. The result is a group of multinucleate compartments in which fusion of the nuclei occurs as described for *G. gnemon*. The compartments are formed first in the vicinity of the egg and around the end of the pollen tube. Consequently at fertilization time one finds this group of cells surrounding the egg and male nuclei and in the remainder of the expanded part of the sac only free nuclei. The latter region becomes filled with cells partly by the division of those already present and partly by the formation of new compartments among the free nuclei.

Meanwhile endosperm has been forming down in the neck of the flask. Here too the process is not the same as in *G. gnemon*. The original divisions accompanied by increase in the cytoplasm, continue until the whole of this part of the sac becomes filled with cytoplasm and nuclei. In other words the vacuole becomes filled. It will be recalled that this part of the sac is much longer and narrower than in *G. gnemon*. The whole neck of the flask then becomes divided into compartments. Figure 60 shows this condition in longitudinal section and figure 61 in transverse section. I could not see any relation between division spindles and walls in this region; in fact the latter appeared to be more in the nature of cleavage walls. Each compartment contains more nuclei than in *G. gnemon*, but fusion takes place in the same way, resulting in the production of uninucleate cells.

At a certain stage in endosperm formation, therefore, one finds cellular tissue throughout the narrow part of the sac and around the fertilized egg in the expanded part. Elsewhere in latter region are free nuclei or vacuoles (see figs. 49 and 52). The whole sac later becomes filled with cells by the division of those already present and by the formation of new ones. Further growth takes place chiefly in the lower end of the sac. Consequently the form of the endosperm

becomes reversed, the large end being below. The nucellus is grad-
ually replaced. The originally expanded part of the sac enlarges
very little, if any, and the nuclei and protoplasm within it disappear.
The cells in this region become largely disorganized by the growth
of the suspensors.

It should be pointed out that considerable development has taken
place in this endosperm before fertilization occurs. While there is no
cellular tissue in the sac at the entrance of the pollen tube, a con-
siderable amount of it develops before the actual fusion of the sexual
nuclei. In fact fertilization does not take place until the narrow part
of the sac is filled with compartments and the group of cells have
been formed in the expanded part of the sac.

It is evident that the process of endosperm formation in *G. sp. 33*
is more primitive than that of *G. gnemon*. In the first place the
whole sac of the former species becomes divided into cells as in typical
Gymnosperms and all the nuclei contribute to endosperm formation,
while in *G. gnemon* only a few of the nuclei contribute to endosperm
formation. In the second place the development of endosperm in
G. sp. 33 has gone much farther before fertilization than in *G. gnemon*.

The important departures from the typical Gymnospermic method
of endosperm formation are three in number: (1) The delay in cell
formation until after the pollen tube has entered, (2) the fusion of
nuclei in each cell, (3) the participation of only a few of the nuclei
in endosperm formation (*G. gnemon*). Now all these departures from
the Gymnospermic method are approaches to the Angiospermic
method. They will be discussed separately.

1. In all other Gymnosperms a cellular endosperm is formed
before the pollen tube enters the archegonium and the egg is fertilized.
In Angiosperms the endosperm never forms until after the pollen
tube enters the embryo-sac though it may begin to develop before
actual fertilization takes place. In Gnetum one never finds endo-
sperm before the entrance of the pollen tube. It is plain therefore
that in this very important respect, Gnetum follows the Angiospermic
method. But in *G. sp. 33* there is a reminiscence of the Gymnospermic
method in that a considerable mass of endosperm is formed before
the actual fusion of sexual nuclei occurs. In *G. gnemon* fertilization
takes place sooner, though I have never seen a fertilized egg before a
few cells were formed. In this connection it should be pointed out
that in Casuarina, one of the lowest of the Angiosperms, Treub (31)

observed a considerable mass of endosperm before fertilization occurred.

2. In typical Gymnosperms there is nothing which resembles the fusion of nuclei which in Angiosperms precedes endosperm development. In typical Angiosperms two nuclei only of the female gametophyte, a micropylar and an antipodal, unite. In Gnetum several nuclei in each compartment unite. The chief differences from Angiosperms are (1) that many fusion nuclei result and (2) that several nuclei unite to form a single fusion nucleus. Nevertheless the essential fact remains that a fusion of nuclei occurs before the endosperm develops. And it should be remembered that in several primitive Angiosperms more than two nuclei unite. Thus in *Peperomia hispidula* Johnston (11) reports that 14 nuclei fuse to form the endosperm nucleus. Therefore one of the two differences between Gnetum and Angiosperms in this connection disappears (and it is significant that of all the Angiosperms, forms like Peperomia should most resemble Gnetum). The other difference, the number of fusion nuclei, loses its importance when we remember that in *G. gnemon*, there is a tendency to reduce the number [see (3) below]. Accordingly it seems a reasonable conclusion that the fusion of nuclei in Gnetum is really a forerunner of that in Angiosperms.

3. In Gymnosperms the whole female gametophyte is endosperm tissue. In Angiosperms endosperm formation involves only two nuclei of the female gametophyte. *G. sp. 33* resembles the Gymnosperms in that all the gametophytic nuclei contribute to endosperm formation. There are many fusion nuclei throughout the sac. But in *G. gnemon* only the nuclei at the bottom of the sac are concerned in endosperm formation. At most ten compartments with their fusion nuclei participate while all the rest of the sac (by far the larger part) has nothing to do with it. Within the genus itself, therefore, there is a marked tendency away from the Gymnospermic condition in *G. sp. 33* toward the more Angiosperm-like condition of *G. gnemon*.

The only other important difference between the endosperm of Angiosperms and that of Gymnosperms is one of which we find no trace in Gnetum, namely, the fertilization of a fusion nucleus by a male nucleus. No male nucleus is concerned with endosperm formation in any species of Gnetum. At this important point, therefore, the resemblance between Gnetum and the Angiosperms breaks down. In other words Gnetum does not throw much light on the

origin of "double fertilization" or "triple fusion" and yet from the
conditions in Gnetum one may well imagine how the phenomenon
arose. It should be remembered that in the embryo-sac before
fertilization there are fusion nuclei and a male nucleus in addition
to the egg and male nucleus which will fuse to form the embryo.
While I cannot confirm Lotsy's statement that the second male
nucleus always functions, I have seen one case in which it did function.
If the second male nucleus fertilized a fusion nucleus in place of an
egg the typical Angiosperm condition would be reached. Further-
more it has been shown that in certain of the lower Angiosperms
double fertilization does not occur.

We have just concluded that in respect to three of the four im-
portant differences between the endosperm of Gymnosperms and
that of Angiosperms, Gnetum resembles the latter. And yet in re-
gard to each of them we find evident reminiscences of the Gymno-
sperm condition. It seems, therefore, a reasonable conclusion that
the endosperm of *Gnetum* is really the type from which that of Angio-
sperms has been derived, and that it in turn has been derived from
the Gymnosperm type.

Moreover the conditions in Gnetum have a distinct bearing on
the morphology of the endosperm in Angiosperms. As is well known
there are two views on this subject: (1) that it is belated gameto-
phytic tissue and (2) that it is really an embryo rendered monstrous
by the introduction of the second female nucleus. In Gnetum there
can be no doubt that it is belated female gametophyte, although
the fusion of nuclei preceding its initiation is difficult to understand.
Indeed, although the conditions are much more primitive in Gnetum
we appear to be no nearer an understanding of the meaning of the
fusion than we are in the case of Angiosperms. We can only fall
back on the old idea that it is in the nature of a vegetative stimulus
to growth. Now in spite of the fertilization of the fusion nucleus by
the second male nucleus in a great many Angiosperms, the endosperm
of the latter group is of the same nature as that of Gnetum. This
is true not only because of the transitions seen in Gnetum but also
because the triple fusion is not always a prerequisite to endosperm
formation in Angiosperms. It follows therefore that the endosperm
of Angiosperms is just as much female gametophyte as is that of
Gnetum and this would be true whether or not the endosperm of
Angiosperms had been derived from that of Gnetum.

11. Embryo

The nucleus of the fertilized egg is large and dense.   Its nucleolus is extremely large and has a distinct cavity in the center.   In *G. gnemon* the fertilized egg is surrounded by a mass of protoplasm and free nuclei (see figure 54), while in *G. sp. 33* it is surrounded by cells of the endosperm (figure 51).   The development of the embryo in *G. sp. 33* will be described first.

The fertilized egg divides two or three times and produces a small group of cells.   These divisions are accompanied by wall formation. There appears to be no definite order in the divisions and no definite arrangement of the cells.   This pro-embryo can usually be readily distinguished from the surrounding endosperm cells by the density of the protoplasm and size of the nuclei and nucleoli.   Figure 74 represents such a pro-embryo against the end of the pollen tube. Each cell then elongates to form a suspensor (figures 75 and 76) which penetrates far towards the bottom of the endosperm.   (It will be recalled that most of the growth of the endosperm occurs in the lower part of the sac.)   These suspensors are very difficult to follow and consequently it is hard to determine their nuclear condition with certainty.   In almost all cases one can find only a single enormously large nucleus in each.   I have, however, seen newly forming sus-pensors with two nuclei and in *G. moluccense* I have seen two large nuclei near the end of an elongated suspensor.   On account of the difficulty of tracing the tortuous suspensors through their whole length, however, it may be that some nuclei were overlooked.   But in any case very few nuclei are present, probably only one normally. If more are formed they must degenerate very quickly.[1]   In the much elongated suspensors the common appearance is a great length of empty tube with a mass of deeply staining protoplasm and a huge nucleus only at the end.   So far as I have observed walls are never formed in this suspensor, and there is very little branching.

The later stages of embryogeny I have seen only in *G. moluccense* and in *G. funiculare* but as in most other respects these species re-semble *G. sp. 33* it is altogether likely that in the latter species the conditions are similar.   In *G. moluccense* the suspensors are very often found outside the endosperm.   They grow either through the latter or just between it and the nucellus.   Sometimes they grow

[1] I regret that in my preliminary note (28) I gave the impression that many nuclei were present.

even beyond the endosperm into the tissue at the base of the nucellus. Figure 97 represents such a condition. I have never seen an embyro developing in this position, they were always within the endosperm. Before the embryo proper is formed the end of the suspensor enlarges and in the next stage that I have observed this swollen end contained four small nuclei (see figure 78). There was no wall separating the swollen end from the rest of the tube. From this structure the embryo is organized in a manner not determined. A young embryo consisting of a small group of cells irregularly arranged is shown in figure 80.

In *G. gnemon* the pro-embryo differs from that of the other species in that only two cells are formed before the development of suspensors. The two-celled stage is shown in figure 70. I cannot state whether or not the dividing wall develops in connection with the division of the nucleus or is in the nature of a cleavage wall. Both cells develop into suspensors (figure 71), which grow down through the empty upper part of the sac to the developing endosperm which they penetrate. Figure 72 represents the suspensors growing towards the endosperm and figure 73 the same thing under greater magnification. I have not observed the later stages in this species.

This account of the embryogeny of *G. gnemon* differs from that of Lotsy in regard to the division of the fertilized egg. Lotsy states that the fertilized egg itself elongates without division to form the suspensor. The explanation of the discrepancy in the two accounts is likely to be found in Lotsy's statement that both male cells always function in fertilization. Apparently what Lotsy considered to be two fertilized eggs, I have called a two-celled proembryo. In support of my view I would call attention to three points: (1) I have more than once observed one fertilized egg alone in a sac (this may, however, be interpreted as the failure of one of the male cells to function; but in that case, the statement that both male cells always function is incorrect); (2) the two cells are always in close contact and have every appearance of having resulted from the division of a single cell; (3) in *G. sp. 33* several cells are undoubtedly formed before the suspensors elongate.

Coulter (7) states that the suspensor within the endosperm is divided by transverse cleavage walls. While this may be true within the endosperm I have observed no such walls before the suspensor reaches that tissue. Furthermore in the other species no such walls

were observed even in the endosperm. Coulter's account follows the embryo-formation farther than I have done, showing that the multinucleate terminal cell becomes divided up by cleavage walls into uninucleate cells.

The most important point about this process of embryo formation is that the free nuclear divisions characteristic of Gymnosperms are eliminated. Division of the fertilized egg in *G. sp. 33* at least, is accompanied by wall formation as in Angiosperms. In another important respect therefore Gnetum takes its place with the Angiosperms. This elimination of the free nuclear stage was foreshadowed in Welwitschia (Pearson, 21) in which only two free nuclei are formed and are then separated by a cleavage wall.

12. GENERAL CONCLUSIONS

The significance of the conditions found in Gnetum has been discussed in connection with the descriptions of the various structures examined. It remains to point out certain general conclusions which may be deduced from a consideration of the evidence as a whole. The conclusions are in respect to the relationships of the Gnetales (*a*) to each other, (*b*) to the Gymnosperms, (*c*) to the Angiosperms and also in respect to the origin of Angiosperms.

(*a*) The evidence shows that the three genera of the Gnetales are widely different in many morphological points and yet are really phylogenetically related. In habit, anatomy, gametophytic structure, endosperm and embryo formation the genera are all very different. Nevertheless in regard to each of these subjects there are essential points which indicate a common phylogeny. For example, the occurrence in the flowering axis of Gnetum of a type of vessel found elsewhere only in Ephedra can be explained only on the basis of a phylogenetic connection. Other examples are the similarity in flower structure and in the early stages of embryo formation, although the details vary greatly in the three genera. If all three are really related it follows from their present great differences that the Gnetales must formerly have been a very large and diversified group. While the three genera can by no means be arranged in a series according to the degree of specialization it is clear that Gnetum is in many respects the most advanced, that in a few respects Welwitschia has progressed furthest, and that Ephedra is nearest to the ancestral Gymnosperm stock.

(*b*) The conditions in Gnetum throw little direct light on the problem of the Gymnospermic relationship of the Gnetales. Being in many respects the most specialized member of the group, it presents little evidence not already revealed in the case of Ephedra and Welwitschia. The evidence concerning the Gymnospermic relationship must naturally be sought in the most primitive member of the group, the genus Ephedra. That evidence has been presented from the gametophytic standpoint by Land (15, 16) and from the anatomical standpoint by the writer (27). From both fields it indicates definitely that the relationship is with the Coniferous Gymnosperms. It is, however, difficult to understand how the primitive bisporangiate Gnetalean flower can have been evolved from anything found in the Coniferales. But there is nothing found elsewhere in the Gymnosperms which offers an easier solution of the origin of this flower.

(*c*) In regard to the Angiospermic relationship it will be recalled that almost every structure described in the preceding pages shows some approach to the Angiospermic condition and that some structures show conditions almost completely Angiospermic. The more important points are: the form of the inflorescence, particularly of the abnormal ones, the arrangement of the parts of the flower, the presence of an ovary with a style, the form of the stamen, the germination of the microspores at some distance from the nucellus, the behavior of the stalk cell, the free-nucleated embryo-sac and absence of archegonia, the organization of eggs, the fusion of nuclei preceding endosperm formation, the reduction in the number of endosperm producing cells, and the absence of free nuclear divisions in the proembryo. That the anatomy is just as clearly Angiospermic is evident from the possession of vessels, broad rays, and companion-cells in the bast. The habit of course is completely Angiospermic. Such a body of evidence can scarcely be ignored or put aside as the result of parallel development. Indeed in applying most of the contrasts ordinarily employed to distinguish Angiosperms from Gymnosperms, it is found that Gnetum would be classified with the higher group. Accordingly the sum of the evidence from all sides seems to lead to the conclusion that Angiosperms are phylogenetically related to Gnetales. This does not mean that any modern member of the Gnetales represents the type from which Angiosperms were derived but that the ancestors of Angiosperms were not far removed from the genus Gnetum.

The only group of plants which rivals the Gnetales in the claim

on the ancestry of the Angiosperms is the Mesozoic Bennetitales. Their claims have been advocated by Arber and Parkin (1) and by Scott (24). According to the latter author they are three in number: (1) the organization of strobili on the same plan as in typical Angiosperm flowers (2) the formation of a fruit enclosing the seeds (3) the exalbuminous nature of the seeds. It is admitted that these are the only points whether of phylogenetic significance or not in which the Bennetitales resemble Angiosperms. In regard to the exalbuminous nature of the seeds it need only be remarked that by no means all Angiosperms have such seeds and certainly this is not the case in many primitive forms. In regard to the formation of fruit enclosing the seeds the claims of Gnetum are even stronger for the fruit of the latter is formed in a much more typically Angiospermic fashion. The organization of the strobili while resembling that of an Angiosperm flower in regard to the arrangement of the mega- and microsporangia is widely different in many important respects: The ovules are stalked; there is nothing resembling a carpel (a most important point); there are sterile scales between the ovules; the microsporophylls with their fern-like branching and numerous sporangia are as different as possible from the Angiosperm stamen. Moreover if we conclude that the Angiosperm flower of the Magnolia type has been derived from the Bennetitales strobilus we encounter the insurmountable difficulty of explaining the occurrence of the simple catkin-like inflorescence on forms admittedly primitive. On the other hand these catkin-like inflorescences relate themselves strikingly to the strobili of Gnetum.

Apart from the organization of the strobili there are many points which seem to preclude the possibility of the Bennetitalean ancestry of the Angiosperms. Some of them are: the Cycadean habit and leaves, all the essential anatomical points (see Thompson, 27), undoubted possession of motile spermatozoa, primitive Gymnosperm condition and absence of anything foreshadowing the Angiosperm condition in gametophytes, endosperm or embryo. Now in all these respects, as has been shown, Gnetum approaches the Angiosperm conditions and in many of them has actually reached those conditions. As between the Bennetitales and Gnetales, therefore, the decision must surely be made in favor of the latter group. Nor is it sufficient to assume with Arber and Parkin (2) that the Gnetales and Angiosperms developed in parallel lines from the Bennetitales. Aside

from the argument that the points just mentioned preclude any possibility of the Bennetitalean ancestry of Angiosperms, the great resemblance between the latter group and the Gnetales are not satisfied by any such theory. Moreover the evidence relates the Gnetales, not to the Bennetitales but to the Conifers.

It remains to remark that the Amentales are the Angiosperms which most closely resemble the Gnetales and appear therefore to be the most primitive order of the phylum. There is the possibility that both Gnetales and Amentales are somewhat reduced as is indicated by the flower structure of the former.

13. SUMMARY

1. *Actual Conditions.*—Abnormal strobili are found in which the flowers are arranged in a spiral, the whole resembling very much a catkin of the Amentales. Vessels of the Ephedra type are present in the axis of the strobilus.

The development of the microsporangium takes place in the usual way. Two layers of parietal cells are formed. There is no endothecium. The tapetum is developed from sporogenous cells. The period of development is very short.

In the development of the megasporangium the three envelopes arise in acropetal succession. The style develops conducting tissue. Two to four mother cells are formed, two or three produce megaspores, and two or three megaspores develop into embryo-sacs.

The usually abortive ovules of the staminate strobili frequently possess embryo-sacs and may produce seeds.

In the male gametophyte no prothallial cells are formed; the microspores frequently germinate in the style at a distance from the nucellus; the stalk cell never passes into the pollen tube; distinct male cells are formed while the tube is on the nucellus.

The female gametophyte develops in typical Gymnospermic fashion until approximately 256 free nuclei are present in *G. gnemon* and 512 in other species. No cell walls are formed before the entrance of the pollen tube. Definite eggs are organized. Two or three gametophytes usually develop in each ovule.

After the entrance of the pollen tube rapid divisions occur in the female gametophyte. Multinucleate compartments are then formed and all the nuclei in each compartment fuse. The further growth of the endosperm occurs by the division of these compartments. In *G. gnemon* only a few fusion nuclei participate in endosperm formation.

In *G. sp. 33* at least fertilization is delayed until after a considerable amount of endosperm is formed.

The fertilized egg divides to form two cells in *G. gnemon* and several cells in other species. Each of these elongates to form a suspensor from the end of which an embryo is formed.

2. *Inferences.*—The strobili of Gnetum are closely related to the catkins of Amentales.

The flowers of Gnetum are reduced from a bisporangiate condition with parts arranged as in typical Angiosperm flowers.

The inner envelope of the ovulate flower is an ovary homologous with that of Angiosperms and bearing a true style.

The outer envelopes are in the nature of a perianth.

The abortive ovulate flowers are homologous in every respect with the fertile flowers.

The male gametophyte is typically Angiospermic except that it has an evanescent stalk cell.

The female gametophyte is typically Gymnospermic in the early stages but distinctly Angiospermic in the later ones.

The fusion of nuclei preceding endosperm formation is a forerunner of that in Angiosperms.

It is not possible to relate the structures of an Angiosperm embyro-sac to archegonia.

The endosperm of Angiosperms is best interpreted as gametophytic tissue.

The proembryo is Angiospermic.

The different genera of the Gnetales are widely different in morphology and yet are phyogenetically connected.

The Angiosperms have been derived from ancestors very much like modern Gnetales. In fact the genus *Gnetum* should probably be classified with Angiosperms.

14. ACKNOWLEDGMENTS

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16. Explanation of Plates II—VII

Plate II

Fig. 1. *Gnetum funiculare.* Abnormal spiral strobilus. *c.*, collar; *f.*, ovulate flower. × 2.

Fig. 2. *G. gnemon.* Abnormal staminate strobilus with many abortive ovulate flowers. *f.*, ovulate flower; *m.*, place of attachment of staminate flowers which have fallen. × 2.

Fig. 3. *G. gnemon.* Part of similar strobilus at a younger stage. *F.*, ovulate flowers; *m.*, staminate flowers. × 5.

Fig. 4. *G. gnemon.* Radial section of group of young flowers from staminate strobilus. *f.*, abortive ovulate flower; *pe.*, perianth; *r.*, central rudiment of staminate flower; *c.*, collar. × 200.

Fig. 5. *G. gnemon.* Similar section of older group. *m.*, microspore mother cells. × 200.

Fig. 6. *G. gnemon.* Young microsporangium. *e.*, epidermis; *a.*, archesporium. × 400.

Fig. 7. *G. gnemon.* Later stage. *e.*, epidermis; *p.*, primary parietal layer; *s.*, sporogenous cells. × 400.

Fig. 8. *G. gnemon.* Later stage. *p.o.*, outer layer of parietal cells; *p.i.*, inner layer. × 400.

Fig. 9. *G. gnemon.* Microsporangium. *ta.*, tapetum; *m.*, mother cells. × 400.

Fig. 10. *G. gnemon.* Microsporangium in stage of tetrad divisions. Letters as before. × 500.

Fig. 11. *G. sp. 33.* Young ovulate flower. *pe.*, perianth. × 100.

Fig. 12. *G. sp. 33.* Young ovulate flower. *o.i.*, outer integument; *i.i.*, inner integument. × 100.

Fig. 13. *G. sp. 33.* Later stage of ovulate flower. *h.*, hairs. × 50.

Fig. 14. *G. sp. 33.* Later stage. *i.i.*, inner integument elongating to form style; *s.*, embryo-sac. × 100.

FIG. 15. *G. sp. 33.* Mature ovulate flower. *pe.*, perianth; *st.*, style; *cav.*, cavity in style; *o.i.*, outer integument; *i.i.*, inner integument; *n.*, nucellus; *s.*, embryo-sac. × 50.

FIG. 16. *Peperomia sp.* Young ovulate flower. *ca.*, carpel. × 50.

PLATE III

FIG. 17. *G. sp. 33.* Longitudinal section of style. *n.*, nutritive layer; *p.*, pollen grains; *p.t.*, pollen tube. × 300.

FIG. 18. *G. sp. 33.* Style. Later stage. Lettering as before. Shows disintegration of nutritive layer. × 320.

FIG. 19. *G. sp. 33.* Young megasporangium. *e.*, epidermis; *a.*, archegonium. × 550.

FIG. 20. *G. sp. 33.* Young megasporangium. *p.*, parietal cells; *m.*, mother cells. × 550.

FIG. 21. *G. sp. 33.* Young megasporangium. Later stage showing one parietal cell divided. × 550.

FIG. 22. *G. sp. 33.* Young megasporangium. Transverse section. × 550.

FIG. 23. *G. sp. 33.* Young megasporangium, showing two mother cells, one of which is dividing. × 550.

FIG. 24. *G. sp. 33.* Young megasporangium, showing one mother cell undivided and two which have divided and produced daughter cells (*d*). × 550.

FIG. 25. *G. sp. 33.* Megaspores and four-nucleate sac. × 550.

FIG. 26. *G. gnemon.* Longitudinal section of young abortive ovulate flower from staminate strobilus. *pe.*, perianth; *n.*, nucellus; *i.i.*, inner integument; *o.i.*, rudimentary outer integument; *s.*, embryo-sac. × 200.

FIG. 27. *G. gnemon.* Transverse section of abortive ovulate flower. *v.b.*, vascular bundles. × 50.

FIG. 28. *G. gnemon.* Young microspore. × 1200.

FIG. 29. *G. gnemon.* Microspore. *t.*, tube nucleus; *g.*, generative cell. × 1200.

FIG. 30. *G. gnemon.* Microspore. Pollination stage. *t.*, tube nucleus; *b.*, body; *st.*, stalk cell. × 1200.

FIG. 31. *G. gnemon.* Microspore. Stalk and generative nuclei in a common cytoplasm. × 1200.

FIG. 32. *G. sp. 33.* Germinated microspore. × 550.

FIG. 33. *G. sp. 33.* Pollen tube entering nucellus. *b.*, body cell; *i.*, tube nucleus; *n.*, nucellus. × 550.

FIG. 34. *G. sp. 33.* End of pollen tube. *m.*, male nuclei; *t.*, tube nucleus. × 800.

FIG. 35. *G. sp. 33.* *G. sp. 33.* Tip of nucellus with pollen grains and tubes. *st.*, stalk nucleus; *p.t.*, pollen tube; *n.*, nucellus. × 300.

PLATE IV

FIG. 36. *G. gnemon.* Young embryo-sac. × 430.

FIG. 37. *G. gnemon.* Two young embryo-sacs from same nucellus. × 430.

FIG. 38. *G. gnemon.* Nearly mature embryo-sac showing mass of protoplasm and nuclei (*m.*) at bottom. × 200.

Fig. 39.   *G. sp. 33.*   Mature embryo-sac and small abortive one.   × 75.

Fig. 40.   *G. gnemon.*   Top of embryo-sac and pollen tube.   *e.*, egg; *t.*, tube nucleus; *m.*, male nuclei.   × 400.

Fig. 41.   *G. gnemon.*   Whole embryo-sac with entering pollen tubes.   Lettering as before.   × 100.

Fig. 42.   *G. gnemon.*   Top of embryo-sac with entering pollen tube.   × 400.

Fig. 43.   *G. sp. 33.*   Embryo-sac with entering pollen tube showing nuclei all dividing.   *p.t.*, pollen tube; *s.a.*, abortive sac; *d.*, dividing nucleus; *e.*, egg. × 300.

Fig. 44.   *G. gnemon.*   Embryo-sac into which pollen tube has penetrated some distance.   × 300.

Fig. 45.   *G. sp. 33.*   Embryo-sac just prior to fertilization.   *e.*, egg; *m.*, male nuclei; *end.*, cells of endosperm.   × 400.

Fig. 46.   *G. gnemon.*   Nucleus of female gametophyte.   × 1200.

Fig. 47.   *G. gnemon.*   Egg nucleus.   × 1200.

Fig. 48.   *G. sp. 33.*   Male nucleus.   × 1200.

PLATE V

Fig. 49.   *G. sp. 33.*   Embryo-sac at fertilization time.   *t.*, tube nucleus; *end.*, endosperm; *s.a.*, abortive sac.   × 215.

Fig. 50.   *G. sp. 33.*   Sexual nuclei in contact.   *e.*, egg; *m.*, male cells.   × 400.

Fig. 51.   *G. sp. 33.*   Sac with fertilized egg.   *f.e.*, fertilized eggs; *end.*, endosperm.   × 400.

Fig. 52.   *G. sp. 33.*   Sac with fertilized egg.   Earlier stage, showing many free nuclei.   × 300.

Fig. 53.   *G. gnemon.*   Whole embryo-sac with fertilized egg.   *f. e.*, fert. egg; *p.*, mass of protoplasm and nuclei surrounding egg; *end.*, endosperm; *n.*, free nuclei. × 100.

Fig. 54.   *G. gnemon.*   Fertilized egg surrounded by mass of protoplasm and nuclei.   × 400.

PLATE VI

Fig. 55.   *G. gnemon.*   Base of embryo-sac showing beginning of endosperm formation.   *w.*, newly formed wall.   × 300.

Fig. 56.   *G. gnemon.*   Multi-nucleate compartments in endosperm formation. × 300.

Fig. 57.   *G. gnemon.*   Endosperm with partly uninucleate and partly multi-nucleate compartments.   × 300.

Fig. 58.   *G. gnemon.*   Endosperm with uninucleate cells only.   A slight amount of growth has taken place by division.   × 300.

Fig. 59.   *G. gnemon.*   Whole embryo-sac with young endosperm.   *p.e.*, pro-embryo.   × 100.

Fig. 60.   *G. sp. 33.*   Whole embryo-sac with young endosperm.   *s.a.*, abortive embryo-sac; *n.*, free nuclei; *p.t.*, pollen tube; *end.*, endosperm.   × 100.

Fig. 61.   *G. sp. 33.*   Transverse section of embryo-sac with multinucleate endosperm.   × 300.

Fig. 62.   *G. sp. 33.*   Wall formation in endosperm.   × 550.

FIG. 63. *G. gnemon.* Endosperm nuclei massed before fusion. × 800.

FIG. 64. *G. gnemon.* Fusion of endosperm nuclei. Disappearing walls represented by dotted lines. × 800.

FIG. 65. *G. gnemon.* The same; later stage. × 800.

FIG. 66. *G. gnemon.* The same. Later stage. × 800.

FIG. 67. *G. gnemon.* The same. Later stage. Shows the numerous nucleoli. × 800.

FIG. 68. *G. gnemon.* The same. Formation of two masses which fuse later. × 800.

FIG. 69. *G. gnemon.* Endosperm nucleus after several divisions. × 800.

PLATE VII

FIG. 70. *G. gnemon.* Pro-embryo of two cells surrounded by protoplasm and nuclei. × 300.

FIG. 71. *G. gnemon.* Suspensors developing from pro-embryonal cells. × 300.

FIG. 72. *G. gnemon.* Suspensors growing towards endosperm. × 50.

FIG. 73. *G. gnemon.* The same; greater magnification. × 300.

FIG. 74. *G. sp. 33.* Pro-embryo of several cells. × 400.

FIG. 75. *G. sp. 33.* Suspensors developing from pro-embryonal cells. × 200.

FIG. 76. *G. sp. 33.* Old suspensors in embryo-sac. × 100.

FIG. 77. *G. moluccense.* End of suspensor with two large nuclei. × 200.

FIG. 78. *G. moluccense.* End of suspensor showing four nuclei which will give rise to embryo. × 200.

FIG. 79. *G. moluccense.* Suspensors outside of and below endosperm. × 100.

FIG. 80. *G. moluccense.* Young embryo. × 50.

APPENDIX

The following list includes the names of the species used together with the name authorities:

> *Gnetum Gnemon* L.
> *Gnetum latifolium* Blume
> *Gnetum moluccense* Miquel
> *Gnetum neglectum* Blue
> *Gnetum ula* Brongniart
> *Gnetum* sp. Borneo—Buitenzorg Garden Records
> *Gnetum* sp. 33—Buitenzorg Garden Records
> *Gnetum* sp. 59—Buitenzorg Garden Records